CORRELATIVE LABEL-FREE TWO-PHOTON FLUORESCENCE MICROSCOPY AND POLARIZED LIGHT IMAGING

Irene Costantini[1], Miriam Menzel[2], Ludovico Silvestri[1], Nicole Schubert[2], Markus Axer[2], Katrin Amunts[2,3], Francesco S. Pavone[1, 4, 5]

[1]European Laboratory for Non-linear Spectroscopy, University of Florence, Italy
[2]Institute of Neuroscience and Medicine (INM-1), Research Centre Jülich, Jülich, Germany
[3]C. and O. Vogt Institute of Brain Research, Heinrich Heine University Düsseldorf, Düsseldorf, Germany
[4]National Institute of Optics, National Research Council, Italy
[5]Department of Physics, University of Florence, Italy
Via Sansone 1, 50019 Sesto Fiorentino, Italy
E-mail : costantini@lens.unifi.it

KEY WORDS: Fluorescence microscopy, polarized light imaging, **l**abel-free, myelin

One of the main goal of connectomics is to reconstruct the neuronal fiber network that exists among brain regions. 3D-Polarized light imaging (3D-PLI) [1] allows to assess fiber orientation and inclination angles in histological brain sections thanks to the birefringent properties of the myelin sheaths. This optical method enables a fast analysis of fixed mouse and human brains without any exogenous labeling. In this work, we employ an integrated dual approach [2] that combines 3D-PLI with two-photon fluorescence microscopy (TPFM) [3] to study the mixture of various fiber orientations within the sample (and voxel) of interest. We exploit the higher axial and radial resolution of TPFM optical sectioning in combination with a specific preparation protocol for myelin autofluorescence to perform the 3D reconstruction of fiber orientation within each brain section. We demonstrate that the correlation between the two methods permits to reconstruct areas of the brain with the possibility of characterizing a specific sector of the sample at high resolution, below micrometer level, without exogenous labeling. Our study shows that the integration of different techniques allows a label-free analysis of brain connectomics, providing a novel tool for an implemented 3D reconstruction of nerve fiber orientations in fixed samples.

[1] M. Axer, K. Amunts, D. Grässel, C. Palm, J. Dammers, H. Axer, U. Pietrzyk and K. Zilles "A novel approach to the human connectome: Ultra-high resolution mapping of fiber tracts in the brain." *NeuroImage* **54**, 1091-1101 (2011).

[2] L. Silvestri, AL. Allegra Mascaro, I. Costantini, L. Sacconi, FS. Pavone "Correlative two-photon and light sheet microscopy." *Methods* **66**, 268-72 (2014).

[3] WR. Zipfel, RM. Williams, WW. Webb., "Nonlinear magic: multiphoton microscopy in the biosciences," Nat Biotechnol. 11, 1369-77 (2003).